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Observations on the Use of GonaCon™ in Captive Female Elk (*Cervus elaphus*).

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Abstract: Overabundant populations of elk (*Cervus elaphus*) raise concern in the western United States because of habitat damage and transmission of *Brucella abortu*s. The objective of this study was to evaluate the contraceptive efficacy of a single-dose GnRH vaccine (GonaCon™) in female elk. For females given 1,000 µg GonaCon™ the percentages that were pregnant for 2005, 2006 and 2007were 14%, 10% and 0% respectively, compared to 10%, 0% and 0% for females given 2,000 µg GonaCon™ and 77%, 75%, and 100% for controls.

Key words: elk, *Cervus elaphus*, GnRH vaccine, GonaCon™, immunocontraception

In previous studies, we demonstrated that the gonadotropin releasing hormone (GnRH) vaccine, GonaCon™ is highly effective for preventing pregnancy in white-tailed deer (*Odocoileus virginianus*) and other species (Miller et al., 2008). Nevada mustangs (*Equus caballus*; Killian et al., 2004; 2006a), domestic and feral swine (*Sus scrofa*; Miller et al., 2003; Killian et al., 2006b), bison (*Bison bison*; Miller et al., 2004a.) as well as several other species (Levy et al., 2004; Miller et al., 2004b; Nash et al., 2004). In female deer and horses, a single intramuscular injection of GonaCon™ was effective for up to 5 years (Killian et al., 2006a). GonaCon™ stimulates the immune system to produce antibodies against GnRH, a small peptide protein produced by the hypothalamus. When sufficient antibody is present, the antibody complexes with and inactivates GnRH, and prevents it from stimulating reproductive hormone and gamete production until the antibody titer declines to an ineffective level. In safety and toxicity studies with female white-tailed deer, GonaCon™ had no adverse effects based on blood chemistry, post mortem and histopathology evaluations (Killian et al., 2006c).

Contraceptive vaccines can be used as a tool to manage overabundant wildlife populations where lethal means of management are not possible or feasible (Fagerstone et al., 2002), such as in national parks or urban or suburban areas. Miller et al. (2004a) and Killian et al. (2006) introduced the concept that brucellosis transmission could be limited by preventing

pregnancy in conjunction with a *Brucella* vaccination campaign in bison and feral swine. Contraception could prevent pregnancy, and thus abortion, of *Brucella*-infected elk until they cleared the bacteria. We undertook the present study to evaluate the contraceptive efficacy of two doses of GonaCon™, given as a single injection to captive female elk.

The study was conducted at the Sybille Wildlife Research Unit of the Wyoming Game and Fish Department (Wheatland, Wyoming, USA; 41º 45.778' N, 105º 22.605' W) from 2004-2007. In February 2002, female elk calves were captured in corral traps at the National Elk Refuge (Jackson, Wyoming, USA) and transported to Sybille. There, elk were housed in 0.4-ha corrals and fed alfalfa hay supplemented with a pelleted ration. Water and a trace mineral block were provided ad libitum. Chronic wasting disease has existed in the Sybille facility for over 15 years and captive cervids often contract the disease, probably from a contaminated environment (Miller et al., 2004c).

The GonaCon™ vaccine consisted of GnRH peptide conjugated to Keyhole Limpet Hemocyanin (KLH; Miller et al.,2004b, 2008) made into an emulsion with AdjuVac™ adjuvant (Pocatello Supply Depot, Pocatello, Idaho USA). In September 2004, 12 females received a 1 ml injection of 1,000 µg GonaCon™, 10 females received a 1 ml injection of 2,000 µg GonaCon™, 13 control females received a 1 ml injection of the adjuvant-buffer emulsion, and two control cows were untreated. All elk were intermingled at random with 1-3 per pen so that there would not be a pen effect. Intramuscular injections were delivered remotely by darts equipped with biodegradable barbs (Pneu-Dart, Inc., Williamsport, Pennsylvania USA).

At the beginning of November 2004, 2005, and 2006 females were grouped with males for breeding. Males were exchanged among the females groups every other week for three months to maximize conception. Blood samples were collected in February or March of 2005,

2006 and 2007 from the jugular vein while females were restrained in a chute. After clotting, the serum was harvested by centrifugation and kept stored frozen at -20C until assay. Serum was used to determine antibody titers to GnRH and progesterone concentration using methods described elsewhere (Miller et al., 2000, 2004b). Assays for pregnancy specific protein B were performed by BioTracking (Moscow, Idaho USA). Their data are reported as either pregnant or not pregnant based on an internal cutoff set by the Bio-Tracking.

Significant differences among treatments and controls for percentages pregnant were determined by Chi Square. Differences between treatments for antibody titers and serum progesterone concentrations were determined by Students "T" test.

Antibody titers for females treated with 1,000 µg of GonaCon™ averaged 1.04×10^5 five months after the immunization. Average titers were maintained for the 1,000 µg group at .78 and $.85 \times 10^5$ in 2006 and 2007 respectively. Females receiving the 2,000 µg dose of GonaCon™ had an average titer of 1.29×10^5 in 2005 which was less ($P = 0.016$) than the average titers of 1.48 and 1.44×10^5 in 2006 and 2007. These data suggest that there may be an advantage of using the 2,000 µg dose of GonaCon™ over the 1,000 µg dose to provide significantly greater titers.

Compared to the control animals, both doses of the GonaCon™ vaccine were highly effective in preventing pregnancy in elk during the three year study ($P < 0.0001$). Annual pregnancy rates were greatest in the control animals where 77% (11/15), 75% (6/8) and 100% (6/6) were pregnant in 2005, 2006, and 2007, respectively. In contrast, 8% (1/12), 10% (1/10), and 0% (0/8) were pregnant in the group receiving 1,000 µg of GonaCon™ and 10% (1/10), 0% (0/8), and 0% (0/6) were infertile in the group receiving the 2,000 µg dose of GonaCon™.

Although antibody titers were greater in females treated with the 2,000 µg dose of GonaCon™, there was no difference in rates of pregnancy between the 1,000 µg and 2,000 µg GonaCon™ groups ($P = 0.697$) over the three breeding seasons studied. This outcome could change in future years if there is a greater decline in titers for the 1,000 µg group compared to the 2,000 µg group. Taken together, these results suggested that a single injection of either dose of GonaCon™ could be used for elk herds where population management by non-lethal means is desired.

Although some progesterone is secreted during the luteal phase of the estrous cycle, maximal serum concentrations of progesterone are produced by the corpus luteum during pregnancy. Control animals, most of which were pregnant when the blood samples were taken in February or March, had greater average concentrations of serum progesterone ($P < 0.0001$) than cows treated with GonaCon™.

A challenge in managing elk in the GYA is that most herd sizes are over management objectives. Because GonaCon™ significantly reduced pregnancies for three breeding seasons, we suggest that it may prove useful in infected elk herds on winter feedgrounds in the GYA by reducing pregnancies and thereby reducing exposure to *B. abortus*.

Of the 37 females which began the study in the fall of 2004, only 20 were alive when blood samples were taken in April of 2007. The cause of death in every instance was CWD, diagnosed by immunohistochemistry. Despite these animal losses, results of this study indicated that both doses of GonaCon™ were effective in preventing pregnancy of elk for at least three years. We conclude that GonaCon use for population management of elk warrants further investigation as part of strategies to control overabundant populations and potentially the diseases associated with these high density populations.

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